5,800,690, issued September 1, 1998 and titled "Variable Control of Electroosmotic and/or Electrophoretic Forces Within a Fluid-Containing Structure Via Electrical Forces;" and 6,001,231, issued December 14, 1999 and titled "Methods and Systems for Monitoring and Controlling Fluid Flow Rates in Microfluidic Systems."

A1

On page 1, please replace the paragraph beginning "These devices are relatively disadvantageous" with the following paragraph:

A2

These devices are relatively disadvantageous because, *inter alia*, they require larger volumes of reactants by virtue of their flow-based design, and fluid control by electro-osmotic and electrophoretic forces typically requires relatively large voltages, which may be dangerous and are difficult to generate in small portable control devices. Control devices for microfluidic devices based on such technologies are larger, at least desktop in size.

On page 2, please replace the paragraph beginning "It is one of the objects of the present invention" with the following paragraph:

A3

It is one of the objects of the present invention to overcome this deficiency in the art and provide methods and systems for controlling micro-droplet-based microfluidic devices that exploits their essential advantages. Because of the structure and properties of such microfluidic devices, the methods and systems of this invention can be implemented in a wide range of embodiments, from entirely handheld embodiments to laboratory embodiments for performing high-throughput reactions and analyses. Further, because of the structure and properties of such microfluidic device, these methods and systems can be controlled by users to perform diverse reactions and analysis in a manner similar to programming a computer.

On page 3, please delete the paragraph beginning "[???? copies".

On page 4, please replace the paragraph beginning "In still further particular aspects of the first embodiment, the step" with the following paragraph:

In still further particular aspects of the first embodiment, the step of opening or closing a controlled passage further includes melting at least one aliquot of a meltable material, wherein the aliquot of the material is positionable for occluding the controlled passage; the step of providing a gas pressure in a passage further includes heating at least one gas micro reservoir communicating with the passage; the step of sensing the presence of a micro-droplet at a position in a passage further includes sensing an indicator of the thermal capacity in a region about the position; the step of sensing the composition of a micro-droplet further includes sending optical signals to the MF device and receiving optical signals returned from the MF device; the request for creating a new micro-droplet from a fluid aliquot in a passage further includes: (i) one or more actuator processing requests to close the passage in order to prevent the fluid aliquot from moving in a reverse direction along the passage, and (ii) one or more actuator processing requests to provide controllable gas pressure in order to pinch a new micro-droplet from the fluid aliquot in a metered manner and to propel the new micro-droplet to in a forward direction to the selected position; and the request for moving one or more new micro-droplets in a passage further includes: (i) one or more actuator processing requests to close the passage in order to prevent the fluid aliquot from moving in a reverse direction along the passage, and (ii) one or more actuator processing requests to provide controllable gas pressure in order to propel the micro-droplet to in a forward direction to the next stable position.

A4

On page 14, please replace the paragraph beginning "The systems and methods of the present invention may" with the following paragraph:

A5

The systems and methods of the present invention may be applied to control general digital microfluidic devices as just described. They are preferably applied, however, to digital microfluidic devices that have the following additional properties

(i) predominately modular and hierarchical structures, and

(ii) of controllability mainly by electrical control signals.

Such preferred digital microfluidic devices, hereinafter called microfluidic "processors" (or simply "processors"), are preferred over general digital microfluidic

devices because a wide range of different processors may be flexibly and easily controlled by a single programmable control system implementing modular and hierarchically structured control methods. Control of a particular microfluidic process of a particular class is then specified by invoking a high-level control module, which hierarchically encapsulates details of low-level control for all microfluidic processors of that particular class.

A5

On page 18, please replace the paragraph beginning "In this embodiment, device monitoring signals are derived" with the following paragraph:

A6

In this embodiment, device monitoring signals are derived primarily from temperature sensitive elements mounted in the device, which preferably generate electrical monitoring signals such as, for example, temperature-sensitive resistive or semiconductor elements. Localized heating may be precisely controlled by sensed temperatures. Gas pressures may then be controlled by controlled localized heating. Local thermal capacity may be monitored by a combination of a temperature sensor with a small heater by measuring temperature responses with respect to a determined quantity of heat. Using local thermal capacity sensors, micro-droplet presence or absence may be sensed because a micro-droplet has a higher thermal capacity than an otherwise empty passage. Other electrical monitoring signals may be generated by, for example, detecting local electrical impedance, which may provide alternative means for detecting micro-droplet presence. Micro-sensors with deformable conductive elements may provide for direct detection of local pressures.

On page 22, please replace the paragraph beginning "Sub-assembly mixing1 mixes two micro-droplets of differing" with the following paragraph:

A7

Sub-assembly mixing1 mixes two micro-droplets of differing constituents, which have been adjacently positioned at the stable position created by the junction of main passage 4 and the side passage to vent1, in the following manner. First, valve3 (and valve1 and valve2) are closed so that the adjacently situated micro-droplets in passage 4 can be propelled toward passage 5. Next, gas pressure is generated by heater 1, or by heater2, or by both, so that the two micro-droplets in

passage 4 are moved to the stable position just beyond the junction of the side passage to vent2. Importantly, the generated pressure is controlled so that the motion is sufficiently rapid to mix the micro-droplets. Finally, the remaining sub-assembly illustrated in Fig. 1, sub-assembly reaction/detection1, which includes valve5, valve6, heater2, o1, o2, and passage 5, operates as follows. After a mixed micro-droplet of the correct composition is positioned in passage 5, this passage is sealed by closing valve5 and valve6. Next, heater3 is controlled to stimulate a reaction in the trapped micro-droplet, and the results of the stimulated reaction are optically detected by radiation conducted by o1 and o2.

A7

On page 22, please replace the paragraph beginning "Fig. 1 also illustrates leads and external connectors for the electrical" with the following paragraph:

Fig. 1 also illustrates leads and external connectors for the electrical and optical signals. For example, control and monitoring leads 8 for valve1 are schematically illustrated as two leads extending from the valve to the microfluidic processor's edge terminating in connectors 10. (A full and complete illustration of a micro-valve preferably has four, or six or more signal leads.) Although leads 8 are illustrated here as substantially straight, in most microfluidic processors with more actuators and leads, leads bend to avoid obstacles and other leads, or are combined where control requirements allow, or crossover each other separated by insulating films. The terminating connectors are preferably standardized, for example, as an array of pins that may be accommodated by an external socket, or, illustrated here, as rounded protrusions along processor edges that may be accepted by mating contacts in a receptacle in a control system. Also, exemplary optic conductors o1 and o2 are illustrated as extending substantially straight from the reaction/detection sub-assembly to optical couplings or connectors 7, also preferably standardized for routine connection to external radiation sources and detectors. Also, these conductors may need to bend or crossover obstacles. Optical conductors may comprise light pipes, optical fibers, or other means for spatial transmission of an optical signal.

A8

On page 45, please replace the paragraph beginning "Heaters are also preferably resistive and configured to controllably" with the following paragraph:

Heaters are also preferably resistive and configured to controllably generate between 0.5 and 1.5 W of heat with a low voltage source. Since a preferred low source voltage is 5-10 V or less, the resistance of the resistive heaters in the range of approximately 15 Ω to 1000 Ω at 25°C (even smaller heaters may be needed for source voltages of less than 5 V). As Fig. 4A illustrates, a heater with a nearby RTD may provide for controlled heating.

A9

On page 47, please replace the paragraph beginning "The configuration of leads 79-82 is one arrangement that provides" with the following paragraph:

A10

The configuration of leads 79-82 is one arrangement that provides independent control of all three heaters with only four directly-routed and non-overlapping control leads. This illustrated arrangement is exemplary. For example, six leads, two for each heater, may be provided instead.

On page 47, please replace the paragraph beginning "Step 87 then deactivates HTR2 and waits until its temperature returns" with the following paragraph:

Step 87 then deactivates HTR2 and waits until its temperature returns within tolerances to T_0, the baseline processor temperature, so that the plug solidifies again. The return to baseline temperature may either be sensed by a sensor or may be assumed after sufficient time delay. After the plug is solidified, step 88 similarly returns the temperature of HTR1 and gas reservoir 75 to baseline. Because the volume of the gas is now greater because of the motion of the plug out of passage 77, a relatively lower gas pressure is present in reservoir 75 at the baseline temperature when the micro-valve is closed than when it is open.

A11

On page 47, please replace the paragraph beginning "The micro-valve opening operation is described with reference to Fig. 6B" with the following paragraph:

The micro-valve opening operation is described with reference to Fig. 6B, which depicts the micro-valve in a closed state, Fig. 6A, which depicts the micro-

A12

valve in an opened state, and Fig. 6D, which depicts the steps of the micro-valve open function. As customary, this function first obtains 90 input parameters. These parameters identify the control and monitoring connectors and indicate a closed stated (otherwise, the function simply exits). First, the function controllably heats HTR2 and side passage 77 to temperature T_2 and HTR3 and controlled passage 78 to temperature T_1. T_1 and T_2 are both above the melting point of the plug, as described above. Plug 76 in controlled passage 78 thereby melts, and, under the influence of the relatively lower pressure in gas reservoir 75 remaining from the micro-valve closing, is drawn back into side passage 77. These heaters are activated for a time delay 91 determined to be sufficient for the plug to move back into side passage 77. Alternatively, where a position sensor for the plug is available (for example, a thermal sensor in association with HTR2), the delay is until movement of the plug is sensed. Finally, heaters HTR2 and HTR3 are deactivated, and step 92 waits until the temperature in the vicinity of the exit passage heater has returned to within tolerances to baseline (either by temperature monitoring or by time delay). Finally, the micro-valve state is marked as closed with the plug now solidified in exit passage 77 and controlled passage 78 unblocked.

A12

On page 52, please replace the paragraph beginning "The metering operation begins, as usual, at step 120, which" with the following paragraph:

A13

The metering operation begins, as usual, at step 120, which identifies the metering components, their states, their arrangement, order, and their signal lines or external connectors. Optional step 121 opens valve1 and valve2 by means of the actuator-level micro-valve functions, if they were not initially open. Next, the metering function waits 122 for the loading of the fluid aliquot from which a micro-droplet is to be metered. Its loading may be indicated by an external manual signal provided to the user equipment (and transmitted to the DAQ board), or may be automatically indicated by completion of robotic loading, or may be provided by an internal sensor can detect the presence of fluid adjacent to hydrophobic region h1 of passage 110. Step 123 then closes valve1 by invoking the micro-valve close function, so that no more fluid may escape out of vent1.